Chemistry of Natural Compounds, Vol. 36, No. 4, 2000

ALKALOIDS OF *Delphinium poltoratskii*

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UDC 547.944/945

Roots of Delphinium poltoratskii *yield the known alkaloids methyllycaconitine, lycoctonine, anthranoyllycoctonine, ajacine, karacoline, and a new alkaloid delpoline, the structure of which was proved by spectral data and a direct correlation with karacoline. It has been found that the roots and the aerial part of the plant can provide raw material for the production of methyllycaconitine.*

Key words: *Delphinium*, delpoline, diterpene alkaloids.

In continuation of our systematic studies of plants that contain diterpene alkaloids [1, 2], we have studied the alkaloid composition of the roots and aerial parts of *Delphinium poltoratskii* Rupr. growing in Kyrgyzstan. Plants of the *Delphinium* genus are rich sources of valuable alkaloids, in particular, methyllycaconitine. Methyllycaconitine possesses curare-like properties and is used in medical practice [3, 4]. It was recently found that methyllycaconitine and its structural analogs interact with certain types of nicotinic acetylcholine receptors of nerve cells of mammals and insects [5, 6]. These compounds stimulated interest because similar interactions are the focus of studies on Alzheimer's disease and the search for highly effective insecticides among them is promising [7, 8].

D. poltoratskii (Ranunculaceae) is a perennial herbaceous plant. It grows in Kyrgyzstan on alpine and subalpine meadows and moraines and is found in Tyan′-Shan, Bishkek, Osh, Talass, and Dzhalalabad regions [9]. The isolation from this plant of the diterpene alkaloids condelphine, delsoline, and delpyrine has been reported [10].

We investigated alkaloids of the roots and aerial parts of *D. poltoratskii* collected during flowering near the village of Kashka-Suu in Chon-Alaisk region of Osh district in Kyrgyzstan.

The aqueous alcohol extract of the roots gave 2.06%; the aerial part, 0.9% of the mass of dry material as alkaloids. Separation of the total alkaloids yielded the known compounds methyllycaconitine, anthranoyllycoctonine, lycoctonine, ajacine, karacoline, and a new alkaloid named delpoline.

The principal alkaloid in the roots and aerial part is methyllycaconitine, the yield of which from the roots is 0.88%; from the aerial part, 0.47% of the dry mass.

Delpoline (**1**) has the composition $C_{22}H_{33}NO_3$, mp 192-195°C (acetone).

The IR spectrum of **1** contains absorption bands of hydroxyls, an isolated double bond, and ethers. The PMR spectrum exhibits signals for protons of a tertiary C–methyl, N–ethyl, methoxyl, and two olefinic protons. The base peak in the mass spectrum of delpoline is $[M - 15]^+$, which indicates that it is a lycoctonine alkaloid without a substituent on C-1 [11]. The olefinic protons are observed at 5.35 ppm as a doublet with spin—spin coupling constant (SSCC) 9 Hz and at 5.86 ppm as a doublet of triplets with SSCC 9 and 3 Hz. Keeping in mind the mass spectrum that indicates that C-1 is unsubstituted and the nature of the splitting of the olefinic protons, the double bond should be located in ring *A* and can occupy position 1,2 or 2,3. The PMR spectrum of 2,3-dehydrodelcosine, which contains a 2,3-double bond, has signals for the olefinic protons at 5.86 (1H, d, J = 9.4 Hz) and 5.90 ppm (1H, dd, J = 9.4 and 3.3 Hz). The olefinic proton on C-2 undergoes additional splitting by H-1β and appears as a doublet of doublets [12]. In our instance, the second olefinic proton, which appears as a doublet of triplets, indicates that a methylene is located next to the double bond.

The PMR spectrum of delpoline has a signal for H-14β at 4.21 ppm (1H, t, J = 5 Hz), which is consistent with an α-hydroxyl on C-14. The spectral data for delpoline are similar to those for karacoline [13]. Comparison of the empirical

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 0009-3130/00/3604-0390$25.00

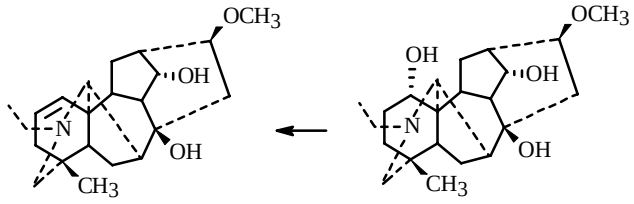
TABLE 1. Chemical Shifts of C Atoms in the ^{13}C NMR Spectra of **1** and **2** (δ, ppm)

C atom	**1**	**2**	C atom	**1**	**2**
1	127.1	72.6	12	28.8	20.0
2	128.8	29.1	13	44.4	44.3
3	40.7	31.5	14	75.9	75.0
4	33.8	33.1	15	41.8	42.5
5	47.8	47.0	16	82.2	82.5
6	24.4	25.4	17	61.5	63.5
7	42.2	45.4	18	26.6	27.8
8	74.5	74.6	19	60.2	60.5
9	46.4	47.0	N-CH_2	48.9	48.6
10	39.9	40.5	CH_3	13.1	13.3
11	47.9	49.1	16-OCH_3	56.4	56.6

formulas of the two alkaloids indicates that they differ by a water molecule, i.e., delpoline contains a double bond in ring *A* instead of a hydroxyl. This was confirmed by studying the ^{13}C NMR spectra of delpoline (Table 1). The multiplicity of the signals was found using the DEPT technique. The signals were assigned by comparison with the spectrum of karacoline (**2**).

The ^{13}C NMR spectra of delpoline (Table 1) argue in favor of the 1,2-location for the double bond. The spectrum lacks a signal for the C-1 methylene. The doublet of C-1 is observed at 127.1 ppm and corresponds to the C atom with the double bond. The weak-field shift (9.2 ppm) of C-3 and the strong-field shift (1.2 ppm) of C-11 also confirm that the double bond is 1,2-situated.

Dehydroxylation of karacoline (**2**) by *p*-toluenesulfonyl chloride in pyridine produced 1-dehydroxy-1,2-dehydrokaracoline as the principal product, which was identical to delpoline. Thus, delpoline has the structure **1**. Delpoline is the first alkaloid with the lycoctonine framework, which contains a 1,2-double bond.



1 **2**

EXPERIMENTAL

Melting points were determined on a Kofler apparatus. Mass spectra were measured on a MS-25 RF (Kratos) GC—MS with direct sample introduction into the ion source. PMR and ^{13}C NMR were obtained on a Bruker AM 500 MHz instrument ($CDCl_3$, 0 - HMDS); IR spectra, on a Perkin—Elmer Model 2000 Fourier-IR spectrometer as KBr pellets.

For chromatography we used KSK silica gel and deactivated aluminum oxide. The purity of the compounds was monitored by TLC using silica gel and aluminum oxide and solvent systems: 1) $CHCl_3$—CH_3OH (10:1 and 100:1) and 2) ether—petroleum ether (1:1).

Isolation and Separation of Total Alkaloids. Air-dried ground roots of *D. poltoratskii* (450 g) were extracted nine times with 80% aqueous ethanol. The ethanol was distilled. The aqueous residue was basicified with Na_2CO_3. The alkaloids were extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated to a volume of 1.5 l and shaken with H_2SO_4 solution (5%) until the alkaloids were completely extracted. The acidic solution was filtered, washed three times with $CHCl_3$, and basicified with Na_2CO_3 with cooling. The alkaloids were exhaustively extracted with $CHCl_3$. Distillation and removal of solvent produced wash (0.11 g) and basic (9.15 g) $CHCl_3$ fractions.

The aforementioned method produced wash (0.09 g) and basic (4.41 g) $CHCl_3$ fractions from the aerial part (500 g) of the plant. The basic $CHCl_3$ fraction of the roots (9.15 g) was dissolved in CH_3OH and treated with methanolic $HClO_4$ until slightly acidic. Homogeneous methyllycaconitine perchlorate (5.87 g) was isolated. The mother liquor after removal of

methanol was dissolved in water, basicified with Na_2CO_3, and extracted with $CHCl_3$. A mixture of bases (2.35 g) was obtained after distillation and removal of solvent. A part of the mother liquor (1.07 g) was chromatographed on a silica-gel column with elution by $CHCl_3$ with continuous addition of CH_3OH. Elution with $CHCl_3$—CH_3OH (100:1) isolated methyllycaconitine (0.11 g) and ajacine (0.06 g). The $CHCl_3$—CH_3OH eluates (50:1) yielded anthranoyllycoctonine (0.07 g) and karacoline (0.03 g). Elution with $CHCl_3$—CH_3OH (20:1) produced lycoctonine (0.17 g). Fractions obtained by elution with $CHCl_3$—CH_3OH (20:1) were combined and rechromatographed on an aluminum oxide column. Elution with benzene—methanol (10:1) yielded lycoctonine (0.09 g); with benzene—methanol (1:1), delpoline (0.06 g).

The basic $CHCl_3$ fraction of the aerial part (4.41 g) was dissolved in CH_3OH and treated with methanolic $HClO_4$ until slightly acidic. Methyllycaconitine perchlorate (2.35 g) was isolated.

Delpoline (1). IR spectrum (ν, cm^{-1}): 3444, 3330, 3032, 2973, 2941, 2883, 2823, 2359, 1669, 1456, 1383, 1350, 1294, 1269, 1241, 1221, 1115, 1090, 1042, 1026, 969, 948, 895, 806, 780, 751, 715, 692, 614, 573, 524, 497.

PMR (δ, ppm): 0.82 (3H, s, 4-CH_3), 1.02 (3H, t, N–CH_2–CH_3), 3.31 (3H, s, OCH_3), 4.21 (1H, t, J = 5 Hz, H-14β), 5.35 (1H, d, J = 9 Hz, H-1), 5.86 (1H, dd, J = 9 and 3 Hz, H-2).

Mass spectrum, *m/z* (%): 359 $[M]^+$ (87), 344 (100), 339 (14), 278 (8), 244 (5), 216 (6), 190 (3), 188 (10), 146 (7), 122 (9), 88 (7).

1-Dehydroxy-1,2-dehydrokaracoline. A cooled (+4°C) solution of karacoline (0.09 g) in dry pyridine (5 ml) was treated with *p*-toluenesulfonyl chloride (0.03 g). The temperature was adjusted to ambient and held there for 10 h. The excess of pyridine was evaporated. The solid was dissolved in water, basicified with Na_2CO_3, and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and evaporated. The product was purified on a deactivated aluminum-oxide column. The fractions obtained by elution with $CHCl_3$—CH_3OH (25:1) were treated with acetone to give 1-dehydroxy-1,2-dehydrokaracoline (0.034 g) that was identical to delpoline in a mixed melting point, TLC, and IR spectrum.

ACKNOWLEDGMENT

We thank Professor M. I. Choudhury from the Chemistry Research Institute of Karachi University, Pakistan, for providing PMR and ^{13}C NMR spectra of delpoline.

REFERENCES

1. M. N. Sultankhodzhaev, in: *Progress in Research of Alkaloid-Bearing Plants* [in Russian], Kh. N. Aripov, ed., Fan, Tashkent (1993), p. 36.
2. F. N. Dzhakhangirov, B. T. Salimov, I. A. Bessonova, and M. N. Sultankhodzhaev, *Khim. Prir. Soedin.*, 841 (1995).
3. P. M. Dozortseva, *Farmakol. Toksikol.*, **22**, 34 (1959).
4. M. D. Mashkovskii, *Medicinal Preparations* [in Russian], Meditsina, Moscow (1984), Vol. 1, 265.
5. P. B. S. Clarke, *Trends Pharmacol. Sci.*, **13**, 407 (1992).
6. J. M. Jacyno, J. S. Harwood, N.-H. Lin, J. E. Campbell, J. P. Sullivan, and M. W. Holladay, *J. Nat. Prod.*, **59**, 707 (1996).
7. D. J. Hardick, I. S. Blagbrough, G. Cooper, B. V. L. Potter, N. Critchley, and S. A. Wonnacoth, *J. Med. Chem.*, **39**, 4860 (1996).
8. K. R. Jennings, D. G. Brown, and D. P. Wright, *Experientia*, **42**, 611 (1986).
9. *Flora of Kirgiz SSR* [in Russian], Frunze (1955), Vol. 6, p. 43.
10. L. I. Brutko and P. S. Massagetov, *Rastit. Resur.*, No. 4, 46 (1986).
11. E. F. Ametova, M. S. Yunusov, and V. A. Tel′nov, *Khim. Prir. Soedin.*, 504 (1982).
12. H. Takayama, T. Okaraki, K. Yamaguchi, N. Aimi, and J. Hadiniwa, *Chem. Pharm. Bull.*, **36**, 3210 (1988).
13. M. N. Sultankhodzhaev, M. S. Yunusov, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, 199 (1973).